Terminating Report on NONR Contract 3985(02) held by Bernard C. Abbott at the University of Illinois

Summary

The research supported by this contract fell into two categories: (1) Studies in Toxins from Marine Algae and (2) Biophysics of Excitable Membranes.

Results

1) Toxic Algae

a) Chrysophycae. Research was carried out on the toxin from Alga Prymnesium parvium. This small unicellular organism grows over a range of slinities from salt water down almost to fresh water. However, it is highly toxic mainly in brackish waters. Waters in which the alga is blooming can be lethal to fish and to cattle which drink the water. Studies have been made during this research on the site of action of the toxin in its lethal role. The substance certainly can pass through the gills of fishes into the blood stream. We have shown that death occurs because of an action in the nervous system and have demonstrated that the toxin blocks the cholinergic synaptic junction. It alsc produces hemolysis of red blood cells but death normally occurs at concentrations where hemolysis is minimal as judged by post portem studies.

A puzzling phenomenon has been noted however in relation to the stability of the poison. When the cells are grown in culture under bright

illumination the toxicity is much less than if the harvesting is carried out at the end of a dark period. In other words the cells grow photosynthetically but the light essential for the photosynthesis also detoxifies the product. The abnormality of the system lies in the fact that the absorption spectrum for the toxin in solution has no significant peaks in the visible region. We have therefore studied the stability of the toxin in relation to the variables light, temperature and aeration.

Cultures were grown and the cells harvested with a continuous flow centrifuge. The toxin was extracted in a series of steps to remove the cell pigments and to obtain maximal purification. The activity of the toxin was calibrated in terms of its hemolytic power on rabbit red blood cells. Solutions of the toxin were mounted in test tubes and exposed to various conditions for 1 hour. a) In light or in darkness, b) at 20°C or 0°C c) in air or in nitrogen.

Combinations of the three variables were used and at the end of the hour the hemolytic power was again determined.

The results indicated very strongly that the detoxification is a photooxidative event not very dependent on temperature, probably as the result of slight pigment impurity.

b) Dinoflagellates. Research continued on the toxic dinoflagellate Gymnodinium breve. This organism is responsible for the Red Tide of the Gulf of Mexico responsible for fish mortality. Culture of this organism

has proved to be very difficult. The growth is very slow and the doubling time is between 8 and 12 days, so that it takes some months to obtain cultures dense enough for harvesting. However, the cells appear to be very fragile. If they are stirred or bubbled the flagellae become detached, so that adequate growth demands a large surface to volume ratio. Furthermore the cells can grow over a range of temperatures from 13° to 26°C but they die if exposed to a sudden change of even 5°C. This has made the research significantly hazardous and dependent on equipment performance over periods of months.

The research on the action of the toxin has indicated a specific action at the synaptic junction by competitive depolarization.

2) Energetics of Excitable Tissue

This research has been carried out throughout the duraction of this contract. The studies have been directed at the fundamental problem of the changes in nerve membranes during an action potential. Much is known of the ionic and electrical changes but little is known of the ultimate mechanism. These studies have therefore been carried out on the energetics of the system, measuring the heat production of the nerve. For this research the temperature changes during an action potential are measured. The results can indicate some of the thermodynamic changes in the system.

For this work nerves with huge numbers of very small uniform fibers are needed. The first studies were carried out with crab leg nerves. This showed that when an action potential occurs there is a heat production followed by a reabsorption of about 80% of that heat. However the time course was difficult to determine because of the uncertain distribution of fiber sizes and velocity of propagation.

Research therefore continued on this contract on this contract on the Rabbit vagus nerve which has a large uniform population of unmedulated nerve fibers. Our studies have shown that these nerve fibers will propogate at temperatures as low as $0^{o}C$ and that heat is given out in the depolarization phase and reabsorbed during repolarization.

The final studies have been made on the olfactory nerves of the garfish. The fibers are extremely uniform, and the action potential at $0^{o}C$ lasts nearly a whole second. Much more important is the fact that although each fiber is only a fraction of a micron in diameter, the action potential can be recorded on a simple pair of external wire electrodes at about 4 mvolt because the fibers discharge so synchronously.

The results show a temperature rise of about $5x10^{-6}{}^{o}C$ followed by a reabsorption of nearly all the heat. However, the action potential was recorded on the same nerve at the same time and the heat reabsorption

set in so rapidly that a detailed analysis is necessary. Because of the very slow propagation velocity it was necessary to make allowance for the wavelength of the action potential.

The evidence that we obtained showed that there was probably an even more interesting set of events than expected. It appears that heat is generated when depolarization occurs, but that absorption occurs in the overshoot. Heat is then given out during the return to zero potential and absorbed in the final repolarization.

This finding is extremely important because it suggests an entropic membrane change. Unfortunately the Office of Naval Research refused to support this research any further and the final analysis remains incomplete.

P-E-A.W.T